

**PATENT COOPERATION TREATY**

From the  
INTERNATIONAL SEARCHING AUTHORITY

To:  
PETER F. CORLESS  
EDWARDS & ANGELL, LLP  
P.O. BOX 55874  
BOSTON, MA 02205

**PCT**

**WRITTEN OPINION OF THE  
INTERNATIONAL SEARCHING AUTHORITY**

(PCT Rule 43bis.1)

**31 JAN 2006**

Applicant's or agent's file reference  60677 (49163)		Date of mailing (day/month/year)
International application No.  PCT/US05/07631	International filing date (day/month/year)  10 March 2005 (10.03.2005)	Priority date (day/month/year)  10 March 2004 (10.03.2004)
International Patent Classification (IPC) or both national classification and IPC  IPC(7): A01N 63/00; C07K 1/00; A01K 67/00 and US Cl.: 424/93.1; 530/350; 800/8		
Applicant  UNIVERSTY OF FLORIDA		

1. This opinion contains indications relating to the following items:

<input checked="" type="checkbox"/>	Box No. I	Basis of the opinion
<input type="checkbox"/>	Box No. II	Priority
<input type="checkbox"/>	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
<input type="checkbox"/>	Box No. IV	Lack of unity of invention
<input checked="" type="checkbox"/>	Box No. V	Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
<input type="checkbox"/>	Box No. VI	Certain documents cited
<input type="checkbox"/>	Box No. VII	Certain defects in the international application
<input type="checkbox"/>	Box No. VIII	Certain observations on the international application

2. FURTHER ACTION

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1bis(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA/ US  Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (571) 273-3201	Date of completion of this opinion  28 December 2005 (28.12.2005)	Authorized officer  Ram R Shukla Telephone No. 571-272-1600
--	---	--

Form PCT/ISA/237 (cover sheet) (April 2005)

WRITTEN OPINION OF THE  
INTERNATIONAL SEARCHING AUTHORITY

International application No.

PCT/US05/07631

Box No. I Basis of this opinion

1. With regard to the language, this opinion has been established on the basis of:  
 the international application in the language in which it was filed  
 a translation of the international application into \_\_\_\_\_, which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).
2. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:
  - a. type of material  
 a sequence listing  
 table(s) related to the sequence listing
  - b. format of material  
 on paper  
 in electronic form
  - c. time of filing/furnishing  
 contained in the international application as filed.  
 filed together with the international application in electronic form.  
 furnished subsequently to this Authority for the purposes of search.
3.  In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

**WRITTEN OPINION OF THE  
INTERNATIONAL SEARCHING AUTHORITY**

International application No.  
PCT/US05/07631

**Box No. V Reasoned statement under Rule 43 bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

## 1. Statement

Novelty (N) Claims 1-13, 16, 17, 22-24, 27-29 YES  
Claims 14, 15, 18-21, 25, 26 NO

**Inventive step (IS)**      **Claims** 5-7,16,17,22-24 and 27-29 **YES**  
**Claims** 1-4,8-15, 18-21, 25 and 26 **NO**

## 2. Citations and explanations:

**Please See Continuation Sheet**

WRITTEN OPINION OF THE  
INTERNATIONAL SEARCHING AUTHORITYInternational application No.  
PCT/US05/07631

## Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

**V. 2. Citations and Explanations:**

Claims 14, 15, 18-21, 25 and 26 lack novelty under PCT Article 33(2) as being anticipated by Kanadia et al. Kanadia teaches a transgenic mouse comprising a deletion of exon 3 of the endogenous *Mbnl1* gene (page 1978, Figure 1). Kanadia also teaches that said mouse exhibits at least one symptom of myotonic dystrophy, namely myotonia and ocular cataracts (page 1979, col. 3, paragr. 2, lines 1-2 and Figure 2); however, Kanadia teaches that said mouse does not develop neonatal muscle weakness that is typically associated with congenital DM1 in humans (page 1980, col. 3, paragr. 1, lines 1-3). Kanadia teaches that the symptoms of said mouse are indicative of a microsatellite repeat expansion disease (i.e. DM types 1 and 2) caused by a microsatellite expansion in a non-coding region of DNA (i.e. within the 3' UTR of the DMPK gene for DM type 1 and within the first intron of the ZNF9 gene for DM type 2; page 1979, col. 1, lines 1-9). Kanadia teaches immunoblot analysis of total spleen protein and thus teaches a cell (i.e. spleen cell) isolated from said mouse (page 1978, Figure 1D). Kanadia teaches that said mouse exhibits abnormal muscleblind proteins in that the *Mbnl1* protein is not expressed in the spleen of said mouse (page 1978, Figure 1D). Kanadia teaches that said mouse exhibits abnormal splicing of *Clcn1* mRNA resulting in *Clcn1* mRNA encoding non-functional CIC-1 protein (page 1979, col. 3, paragr. 2, line 1 to page 1980, col. 1, line 6). Kanadia also teaches that said mouse exhibits similar abnormal splicing of *Tnnt2* and *Tnnt3* mRNA (page 1980, col. 2, paragr. 1).

Claims 1-4 and 8-13 lack an inventive step under PCT Article 33(3) as being obvious over the prior art as applied in the immediately preceding paragraph and further in view of Miller and Hartigan-O'Connor. Kanadia does not teach a method of treating a disease associated with aberrant microsatellite expansion comprising administering nucleic acid encoding *Mbnl1* and does not teach a pharmaceutical composition comprising recombinant adeno-associated virus containing a transgene that encodes *Mbnl1*. Miller teaches a model for DM1 wherein microsatellite-containing mutant DMPK mRNA sequesters MBNL protein from its normal RNA-binding sites (page 4446, col. 1, lines 6-11 and page 4445, Figure 7). Miller further teaches that a causative agent in DM1 may be "sequestration of (CUG)n-binding proteins (i.e. MBNL proteins) on mutant DMPK RNAs and depletion from other transcripts that require these proteins for normal gene expression" (page 4440, col. 1, lines 4-8). It would have been obvious to one of ordinary skill in this art to combine the teachings of Kanadia and Miller to provide the claimed method of treating a disease associated with aberrant microsatellite expansion comprising administering nucleic acid encoding MBNL protein. Further, it would have been obvious to one of ordinary skill in this art to formulate a pharmaceutical composition of a nucleic acid encoding MBNL protein for use in said method by constructing a recombinant adeno-associated virus (rAAV) containing a transgene that encodes MBNL protein. It was well known in this art at the time of the invention that rAAV could be used in pharmaceutical compositions to practice gene therapy. For example, Hartigan-O'Connor teach the benefits of rAAV vectors in general (page 230, col. 1, paragr. 3 to page 231, col. 1, line 2) and specifically in relation to delivery of therapeutic genes to dystrophic muscles (page 225, col. 2, line 1 to page 227, col. 2, paragr. 2 and see entire document). In summary, it would have been obvious to one of ordinary skill in this art to combine the teachings of Kanadia, Miller and Hartigan-O'Connor to provide the claimed method of treating a disease associated with aberrant microsatellite expansion comprising administering a rAAV containing a transgene encoding MBNL proteins.

Claims 5-7, 16, 17, 22-24 and 27-29 meet the criteria set out in PCT Article 33(2)-(3), because the prior art does not teach or fairly suggest: the claimed method of treatment wherein treating comprises reversing the misplicing of the genes encoding amyloid beta precursor protein, NMDA receptor or microtubule associated protein tau; a transgenic mouse comprising a deletion of exon 3 of the

WRITTEN OPINION OF THE  
INTERNATIONAL SEARCHING AUTHORITY

International application No.  
PCT/US05/07631

**Supplemental Box**

In case the space in any of the preceding boxes is not sufficient.

endogenous Mbnl1 gene, wherein said mouse exhibits symptoms typical of a disease associated with aberrant microsatellite expansion in humans, wherein the symptoms of said mouse comprise muscle weakness and ocular cataracts, wherein the disease associated with aberrant microsatellite expansion in humans is caused by a microsatellite expansion in a coding region of DNA, wherein said mouse exhibits loss of functional amyloid beta precursor protein, NMDA receptor or microtubule-associated protein tau; or a method of using a transgenic mouse comprising a deletion of exon 3 of the endogenous Mbnl1 gene for screening compounds useful in the treatment of diseases associated with aberrant microsatellite expansion.

Claims 1-29 meet the criteria set out in PCT Article 33(4), and thus have industrial applicability because the subject matter claimed can be made or used in industry.